

# Translating kinetic selectivity profiles into early safety guidance

## Residence time is a predictor of *in vivo* selectivity

One of the grand challenges in kinase drug discovery is the design of small-molecule inhibitors with selectivity profiles that will ultimately be efficacious in the clinic. Historically, potency-based selectivity assays has been used to determine the potential off-target interactions. However, in recent years there has been increasing recognition that the lifetime of the drug-target complex, rather than affinity alone, can dictate efficacy and safety in patients. Consequently, the evaluation of *in vitro* residence time data for a drug complexed to the primary and collateral targets provide a better understanding of the *in vivo* drug behavior.<sup>1,2</sup>

The development of selective or multitargeted kinase inhibitors depends on the particular disease to be treated. In cancer therapy, single kinase inhibitors will offer a greater chance of effective treatment in tumors that rely on one dominant oncogene for growth and survival. Nonetheless, multikinase inhibitors will be more effective when several kinases contribute to carcinogenesis.

It is important to bear in mind that selectivity is not static in human body. Selectivity evolves over the course of treatment as a function of the temporal binding between the drug and the main and secondary targets. For this reason, designing drugs with the desirable selectivity profiles requires not only an appropriate tuning of binding selectivity but also the modulation of kinetic selectivity.<sup>3</sup>

Over the course of dosing, a drug showing long residence time when bound to its main target and short residence times for secondary targets exhibits temporal target selectivity. Safety and tolerability will considerably improve if the intrinsic toxicity of the drug is minimal. On the other hand, a drug that display a long residence time against a secondary toxicity-mediating target will result in safety issues.

Therefore, the early assessment of kinetic selectivity is crucial to select drugs with the suitable safety profiles.

**Enzymlogic offers the analysis of the compound residence time to the kinase panel of interest using the LanthaScreen® kinase binding technology and our proprietary methodology.**

### FEATURES & BENEFITS

---

- Determination of affinity and residence time in one assay format
- Prediction of the *in vivo* pharmacological selectivity profile
- Identification of potential new uses
- 240 ready-to-use assays
- Quantify binding to inactive or low activity kinases
- HTS format
- Rapid turnaround time: ≤ 2 weeks
- Accurate and reproducible data

## Kinetic selectivity profiling, a successful approach for the discovery of novel drugs

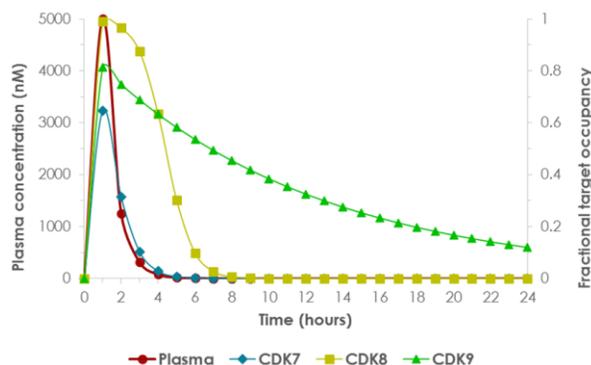
Compounds	$K_i$ (nM)			Residence time (min)		
	CDK7 Cyclin H MNAT1	CDK8 Cyclin C	CDK9 Cyclin T1	CDK7 Cyclin H MNAT1	CDK8 Cyclin C	CDK9 Cyclin T1
Sorafenib	- <sup>a</sup>	30	-	-	55	-
Flavopiridol	8	5	0.4	0.5	15	11
compound 1	2731	45	1152	0.6	17	>360
compound 2	-	39	-	-	1	-
compound 3	405	33	240	<0.5	5	<0.5
compound 4	347	0.8	81	3	12	5

**Table 1. Inhibition of three close-related CDKs by two reference compounds and four compounds from the same chemical series.**  $K_i$  and residence time values were simultaneously determined using a competition kinetic binding assay. 5 nM CDK7, 2 nM CDK8 and 2 nM CDK9 were incubated with 150 nM, 10 nM and 30 nM of ATP-competitive tracer 236 respectively and increasing concentrations of the compounds. The tracer association to kinases was monitored over time. <sup>a</sup> No inhibition.

**Kinetic selectivity profiling is a powerful tool to identify off-target interactions. This approach helps to select drugs with the suitable safety profiles and promotes the discovery of new therapeutic indications.** This phenomenon is exemplified with inhibitors of CDK8/Cyclin C. The binding and kinetic selectivity profile of these compounds have been evaluated against CDK7, CDK8 and CDK9, three close-related CDKs that regulates transcription by phosphorylating RNA polymerase II.

Table 1 shows the affinity and residence time values of reference compounds and four inhibitors from the same chemical series. Taking into account affinity measurements, we can conclude that Sorafenib, compound 1, compound 2 and compound 4 are specific and highly potent CDK8 inhibitors ( $K_i$  values ranging from 0.8 to 45 nM). Such an analysis, however, does not reflect the fact that compound 1 binds to CDK9 in a pseudoirreversible manner. Thus, Kinetic selectivity profiling, and not potency-based selectivity profiling, identify CDK9 as the main target for compound 1.

**Residence time has a dramatic effect on drug efficacy and selectivity *in vivo*.** In human body, drug



**Figure 1. Residence time determines drug-target selectivity.** The pharmacokinetic/pharmacodynamic analysis of compound 1 displays how residence time rather than affinity dictates clinical efficacy and target selectivity over the time course of the treatment. The graph shows the concentration of compound 1 in systemic circulation (●) and the fractional occupancy of CDK7 (◆), CDK8 (■) and CDK9 (▲) for compound 1 after dosing.

efficacy and duration of the pharmacological action do not depend exclusively on affinity. They are also influenced by the residence time of the drug-target complex, the drug concentration in systemic circulation and drug metabolism.

Figure 1 illustrates the effect of residence time on drug efficacy and target selectivity. Affinity and residence time values reported in Table 1 are considered to simulate the *in vivo* performance of compound 1 against CDK7, CDK8 and CDK9. Further, we assume a maximal plasma concentration of 5  $\mu$ M 30 minutes after dosing and an elimination half-life of 30 minutes. Over this time course, the efficacy and selectivity of the compound will depend on the fractional occupancy of each CDK at each time point. At 2 hours after administration 25% of the compound 1 remains in plasma. At this time point, nearly 96% and 75% of CDK8 and CDK9 are inhibited whereas only 31% of CDK7 is occupied by compound 1. At 8 hours, the compound has been almost eliminated from plasma and the percentage of target occupancy is less than 0.1% for CDK7 and CDK8. In contrast, the slow dissociation of compound 1 from CDK9 results in a sustained target occupancy.

CDK9 is still 45% inhibited even though the free compound 1 concentration is well below its  $K_i$ .

The analysis of Figure 1 demonstrates how residence time has a dramatic effect on target occupancy and selectivity when the drug concentration in the body fluctuates over the  $K_i$  for the given target. CDK9, a low affinity target (1152 nM) with a long compound residence time (>6 hours) is more efficiently inhibited

over the course of dosing than CDK8, a high binding affinity target (45 nM) with shorter residence time (17 minutes).

In conclusion, the selection of lead compounds based on their dissociative half-lives from primary and secondary targets is far more relevant for the ultimate efficacy and safety of the compounds than the determination of their potencies.

## References

1. Copeland RA, Pompliano DL, Meek TD. (2006) Drug-target residence time and its implications for lead optimization. *Nat Rev Drug Discov* 5(9):730-739.
2. Tummino PJ, Copeland RA (2008) Residence time of receptor-ligand complexes and its effect on biological function. *Biochemistry*. May 20;47(20):5481-92.
3. Huggins DJ, Sherman W and Tidor B. (2012) Rational Approaches to Improving Selectivity in Drug Design. *J Med Chem* 23;55(4):1424-44.

To learn more about our kinetic selectivity services visit [www.enzymlogic.com](http://www.enzymlogic.com) or contact us at [info@enzymlogic.com](mailto:info@enzymlogic.com)